A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Size-dependency of leaf physiological characteristics and nitrogen allocation in *Robinia pseudoacacia* and *Cornus controversa*

아까시나무와 층층나무의 흉고직경에 따른 잎의 생리적 특성과 질소 분배 특성의 변화

AUGUST 2017

DEPARTMENT OF FOREST SCIENCES
GRADUATE SCHOOL
SEOUL NATIONAL UINVERSITY

MINJEE PARK

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Size-dependency of leaf physiological characteristics and nitrogen allocation in *Robinia pseudoacacia* and *Cornus controversa*

UNDER THE SUPERVISION OF
PROFESSOR HYUN SEOK KIM

SUBMITTING A MASTER'S THESIS
JULY 2017

MAJOR IN FOREST ENVIRONMENTAL SCIENCE
DEPARTMENT OF FOREST SCIENCES
SEOUL NATIONAL UNIVERSITY

MINJEE PARK

APPROVED AS A QUALIFIED THESIS OF
MINJEE PARK
JULY 2017

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Abstract

Leaf traits such as leaf mass per area (LMA), leaf nitrogen (N) concentration, and its allocations are good indicators of physiological and structural characteristics of tree. Understanding these leaf-level traits of different size trees is important for analyzing their responses and predicting their effects on forest ecosystem structure and function. This study investigated the patterns of size (diameter at breast height, DBH) dependent changes in leaf traits of two species with different life-history strategies. In particular, the partitioning of leaf N into the different pools of the photosynthetic and structural apparatus of the species was analyzed. Crown top leaves from thirty-seven *Robinia pseudoacacia* L. (1.3 - 41.3 cm DBH), a light-demanding pioneer and N-fixing species, and fifty-two *Cornus controversa* Hemsl. (0.8 - 48.8 cm DBH), an intermediate shade-tolerant and mid-successional species were collected for the analysis. LMA of two species increased linearly with DBH, while leaf N concentration decreased nonlinearly in a concave and convex shape for *R. pseudoacacia* and *C. controversa*, respectively. Photosynthetic capacities (maximum carboxylation and maximum electron transport rate) of *R. pseudoacacia* stayed relatively constant over the entire DBH range, while those of *C. controversa* had peak values at intermediate DBH. For the leaf N allocation, N concentration in ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (N_R), bioenergetics (N_B), and cell wall (N_W) were higher in *R. pseudoacacia* than *C. controversa*. In contrast, N concentration of light-harvesting (N_H) was higher for *C. controversa* than that of *R. pseudoacacia*. As DBH increased, structural N (N_W) showed concave

patterns for both species. On the other hand, photosynthetic N (N_R, N_B, N_H) in *R. pseudoacacia* and N_B in *C. controversa* linearly decreased, but N_H of *C. controversa* decreased nonlinearly and N_R of *C. controversa* showed convex pattern reaching maximum value of 0.303% about at 6 cm. Our research revealed pronounced changes of leaf traits with tree size reflects the differences in life-history strategies, implying that trees alter their N allocation within the photosynthetic and structural apparatus in leaf according to the life history strategies as they grow.

Keywords: *Robinia pseudoacacia*, *Cornus controversa*, leaf functional traits, size-dependence, leaf mass per area, leaf nitrogen, nitrogen allocation

Student ID: **2014-22820**

Table of Contents

Abstract i

Table of Contents iii

List of Tables v

List of Figures vi

1. Introduction 1

2. Materials and Methods 5

2.1. Study sites 5

2.2. Sampling 6

2.2.1. Tree sampling 6

2.2.2. Leaf and soil sampling 7

2.3. Leaf gas exchange 8

2.4. Crown exposure 9

2.5. Chemical assay 9

2.5.1. Determination of LMA, leaf N and C 9

2.5.2. Determination of leaf N content in Rubisco 10

2.5.3. Determination of leaf N content in bioenergetics 10

2.5.4. Determination of leaf N content in chlorophyll 10

2.5.5. Determination of leaf N content in cell wall 11

2.6. Statistical analysis 12

3. Results 13

3.1. Distribution of DBH, height and crown exposure 13

3.2. Soil N concentration **15**

3.3. Comparisons of leaf morphological and physiological traits **17**

3.4. Influence of crown exposure **19**

3.5. The size-dependency of leaf morphological and physiological traits **21**

3.6. N allocation characteristics **23**

4. Discussion **26**

4.1. Influence of soil N concentration on leaf traits **26**

4.2. Species-specific characteristics of leaf traits **27**

4.3. Size-related changes in leaf traits **28**

4.4. Leaf N allocation **29**

5. Conclusion **30**

References **31**

Appendix 1 **40**

Abstract in Korean **41**

Lists of Tables

Table 1. Results of multiple regression analysis of leaf traits variation as a function of tree size (DBH) and crown exposure. Parameter estimates and results (*P* values) in *R. pseudoacaia* and *C. controversa* are shown. Second-order polynomial terms were included in the analysis when traits exhibit significant DBH^2. *P* values in bold are significant at $P < 0.05$. 20

Lists of Figures

Figure 1. Location of research site in Mt. Taehwa (Google image). Red and yellow circles represent *R. pseudoacacia* and *C. controversa*, respectively. Blue and black crosses in circles represent locations from which soil samples were collected. 6

Figure 2. Distribution of the (a, b) DBH, (c, d) height and (e, f) crown exposure for *R. pseudoacacia* and *C. controversa*, respectively. The crown exposure categories were divided into 9 from under shadow as 1 to fully exposed to the sun as 5 with 0.5 interval as follows: 1, trees without direct light either vertically or laterally; 1.5, trees with low lateral light exposure; 2, trees with medium lateral light exposure; 2.5, trees with high lateral light exposure; 3, co-dominant trees with < 50% of upper crown exposed to vertical light; 3.5, co-dominant trees with > 50% of upper crown exposed to vertical light; 4, dominant trees receiving full vertical and some lateral light, < 50% exposed within 90° inverted cone encompassing the crown; 4.5, dominant trees receiving full vertical and considerable lateral light, > 50% exposed within 90° inverted cone encompassing the crown; 5, emergent trees with crown completely exposed. 14

Figure 3. Differences in (a) total N concentration (in % of dry mass, DM), (b) nitrate N (NO_3-N) concentration (mg kg^{-1}), and (c) ammonium N (NH_4-N) concentration (mg kg^{-1}) of soil between under the crown of *R. pseudoacacia* (*R. p*) and *C.controversa* (*C. c*). The different letters within a panel denote significant differences between group mean values ($P < 0.05$). Error bars

represent the standard error. 16

Figure 4. Differences in (a) LMA (g m^{-2}) , (b) total N concentration (in % of dry mass, DM), and (c) C:N ratio of leaf between *R. pseudoacacia* (*R. p*) and *C.controversa* (*C. c*). The different letters within a panel denote significant differences between group mean values ($P < 0.05$). Error bars represent the standard error. 17

Figure 5. Differences in (a) maximum carboxylation rate (V_{Cmax}) and (b) maximum electron transport rate (J_{max}) between *R. pseudoacacia* (*R. p*) and *C.controversa* (*C. c*). The different letters within a panel denote significant differences between group mean values ($P < 0.05$). Error bars represent the standard error. 18

Figure 6. Leaf morphological and physiological traits as a function of tree DBH for *R. pseudoacacia* (*R. p*) (circle) and *C. controversa* (*C. c*) (filled circle). Leaf traits include (a) LMA, (b) leaf total N, (c) C:N ratio. Trend lines represent leaf traits-DBH relationships based on regression analysis performed on log-log transformed data. 21

Figure 7. Relationships between (a) V_{Cmax} and (b) J_{max} and tree DBH for *R. pseudoacacia* (*R. p*) (circle) and *C. controversa* (*C. c*) (filled circle). Trend lines represent leaf photosynthetic parameters-DBH relationships based on regression analysis performed on log-log transformed data. 22

Figure 8. Differences in (a) N in Rubisco (in % of dry mass, DM), (b) N in bioenergetics (% DM), (c) N in light harvesting (% DM), and (d) N in cell wall (% DM) between *R. pseudoacacia* (*R. p*) and *C. controversa* (*C. c*). The different letters within a panel denote

significant differences between group mean values ($P < 0.05$). Error bars represent the standard error. 24

Figure 9. Size-dependent changes on N allocation characteristics (in % of dry mass, DM) for *R. pseudoacacia* (*R. p*) (circle) and *C. controversa* (*C. c*) (filled circle). (a) N in Rubisco, (b) N in bioenergetics, (c) N in light harvesting, and (d) N in cell wall for each species. Trend lines represent leaf N allocation characteristics-DBH relationships based on regression analysis performed on log-log transformed data. 25

1. Introduction

Tree species with different life history strategies have different morphological and physiological traits and their traits change differently as they age (Adler et al. 2014; Bond 2000). These differences in traits lead to different responses and influence to surrounding ecosystems and resulting in different pathways of succession (Garnier et al. 2004; Gower et al. 1996; Lavorel and Garnier 2002; Visser et al. 2016). Therefore, proper understanding of traits changes with age and/or size for species with various life history strategies is important for the appropriate prediction of ecosystem responses and changes to climate change. Especially, many current models, which predicts the dynamics of forest ecosystem, assume no changes in leaf traits with their size, and the inclusion of functional traits changes related to the growth of tree will be essential to improve model predictions (Botkin et al. 1972; Sendall and Reich 2013).

Leaf functional traits, which are defined as morphological, biochemical, physiological, and phenological characteristics of leaves, are good indicators of tree's response and/or influence on forest community structure and ecosystem function (Lundholm et al. 2015; McGill et al. 2006; Violle et al. 2007). Among leaf functional traits, leaf mass per area (LMA), leaf nitrogen (N) concentration, and photosynthetic parameters (V_{Cmax} and J_{max}) are some of key traits in plant growth (Evans 1989b; Lambers and Poorter 1992). Particularly, N is one of the most important nutrition elements in determining the functional trait of a plant because it composes plant functional proteins, structural proteins and photosynthetic machinery. Furthermore, N is one of the most important

limiting resources for plant growth in nature, and most leaf N is allocated to photosynthesis (Evans 1989a). Therefore, plants are required to allocate the N into different organelles efficiently. Small changes in N allocation can greatly influence photosynthetic capacity, and therefore plant performance. Plant also use N that is not allocated to photosynthesis apparatus structurally in cell walls, a component of plant defense and chemical defenses (Showalter 1993).

Investigations on N allocation have been conducted to find out how plants to invest the important nutrition element to apparatus as the efficient use of N is believed to contribute to fitness of the plant (Guan and Wen 2011; Hikosaka 2005; Onoda et al. 2004; Takashima et al. 2004). A hypothesis put forward by Field and Mooney (1986) suggest that there may be a trade-off between investing N in photosynthetic proteins such as Ribulose-1,5-bisphosphate carboxylase (Rubisco) vs compounds required for longevity. Takashima *et al*. (2004) questioned why some species allocate less N to photosynthetic proteins to have low photosynthetic capacity per unit N. The study revealed that evergreen *Quercus* species with a longer life-span increase N allocation to cell walls and reduced allocation to photosynthesis (growth), showing opposite N allocation characteristics in deciduous *Quercus* species. A similar trade-off in N partitioning between photosynthetic protein (Rubisco) and cell wall proteins was found to be the reason for an intraspecific variation in photosynthetic N use efficiency in *Polygonum cuspidatum* (Onoda et al. 2004).

However, there is considerable uncertainty in the influences of tree size and/or age on leaf traits. Although rates of tissue-level function have been

hypothesized to decline as trees grow older and larger (Fredericksen et al. 1996; Koch et al. 2004; Kolb and Stone 2000; Niinemets 2002; Thomas and Winner 2002), information on leaf trait changes related to tree-specific growth are relatively rare. In particular, quantitative characterization of changes in leaf traits that occur throughout the entire life stage are rarely studied (Niinemets 2002; Rijkers et al. 2000; Thomas 2010). Recent studies reported that size-dependent change on some leaf traits shows nonlinear patterns and the patterns differ among species with different life history strategies (Martin and Thomas 2013; Sendall and Reich 2013). For example, all species in those studies showed that LMA increased monotonically with increasing tree size. However, leaf N of the species show various patterns, increasing, hump-shaped and U-shaped pattern with tree size. This suggested that leaf traits depend not only with the tree size, but also with species. Moreover, there is a lack of detailed study on why leaf N content vary with tree size and why the pattern of the variation is different among species with different life-history strategies. Therefore, I analyzed the change of N allocation with size, this will give us insight for how tree changes their leaf morphology and function with tree size.

Robinia pseudoacacia L. (*R. pseudoacacia*) and *Cornus controversa* Hemsl. (*C. controversa*) were selected for this study because it is expected that they have different life-history strategies and leaf functional traits in particular and they were commonly found across a wide range of tree size at study site. *R. pseudoacacia* was introduced from North America in the 19th century, and is widely planted across South Korea in the 1960s (Korea Forest Service, 1997). This species exhibits the characteristics of large gap species, which are

generally shade-intolerant, disturbance-adapted, and fast-growing (Hobbs 2000; Hobbs and Huenneke 1992; Kowarik 1999; Meffe et al. 1997). *C. controversa* is distributed in Korea, temperate Japan, and subtropical regions of China and is one of main broadleaved deciduous species in Korea (Cho et al. 2000). *C. controversa* is light-demanding species, but has an ability to tolerate low light levels. This species is classified as the mid-successional species (Cornelissen 1993).

The purposes of this study are 1) to analyze the leaf functional traits such as LMA, leaf N and its allocation of two species, *R. pseudoacacia* and *C. controversa* with different ecological and successional status, 2) to find out how these functional traits of the two species change with their size, and 3) to verify the effects of light environment on these size-dependent changes.

2. Materials and Methods

2.1. Study site

This study was conducted at Mt. Taehwa, Seoul National University Forest located in Gwangju, Gyeonggi Province, Korea (37°18'0.5" - 37°19'20.2"N, 127°17'1.3" - 127°18'38.2"E, Figure 1) from late August to mid-September of 2016. Mt. Taehwa belongs to the temperate forest zone; the annual mean air temperature of 2016 was 12.33°C, with a maximum temperature of 33.16°C in August and a minimum temperature of -18.14°C in January and the annual rainfall was 928.2 mm which were measured near flux tower. According to the USDA soil classification system, soils in study sites are classified as sandy loamy, in the mesic family of Typic Dystrudepts (Buol et al. 2011). Soil parent material was an eluvium of metamorphic rock, such as biotite and granite gneiss (Sohng et al. 2014). The University Forest consists of plantation and natural forests. The plantation tree species are *Pinus koraiensis* and *Larix kaempferi* and the entire area is about 300 ha. The natural broad-leaved forests of 497 ha are dominated by oaks (*Quercus acutissima*, *Quercus variabilis* and *Quercus mongolica*). Other major species include *Fraxinus rhynchophylla*, *Styrax obassia*, *Betula davurica*, *Prunus sargentii* and *Cornus controversa* and so on (Choi et al. 2001; Sohng et al. 2014).

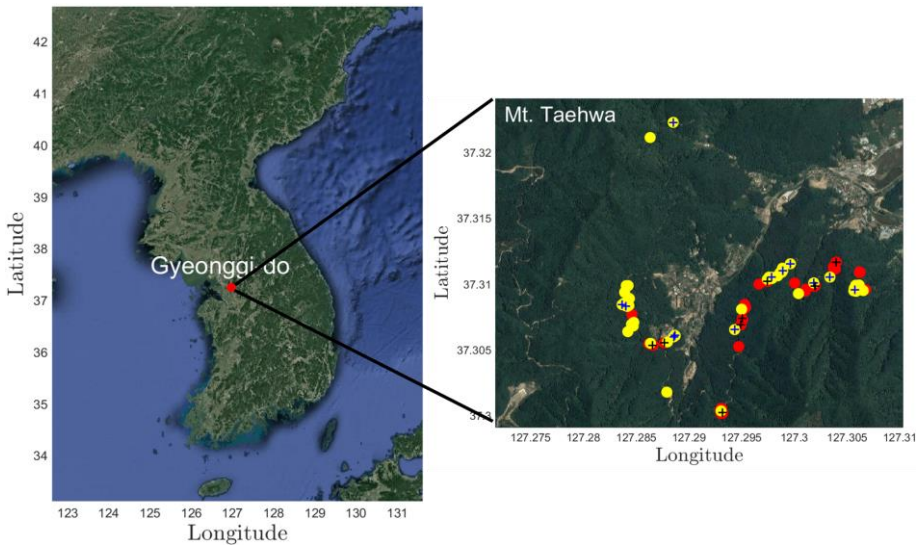


Figure 1. Location of research site in Mt. Taehwa (Google image). Red and yellow circles represent *R. pseudoacacia* and *C. controversa*, respectively. Blue and black crosses in circles represent locations from which soil samples were collected.

2.2. Sampling

2.2.1. Tree sampling

Thirty-seven *R. pseudoacacia* and fifty-two *C. controversa* individuals across a range of tree sizes in DBH and height were selected for analysis. All sample trees were chosen near forest road since both species occur frequently in areas with sufficient light availability (*R. pseudoacacia* individuals were closer to the forest road than *C. controversa* individuals) and its accessibility to leaves at the top of the crown for leaf sampling was considered. In addition, individuals growing in small elevation range (200 - 342 m above sea level) during short sampling campaign (~20 days) were used to reduce spatial and temporal heterogeneity of the samples (Hoch et al. 2002; Martin and Thomas 2013). In order to ensure spatial interspersion of sample

trees of a given species, all sample trees had no overlapping in their crowns one another. This would minimize the influence of other nearby sample trees.

2.2.2. Leaf and soil sampling

Branches from top crown of sample trees were cut down using a telescoping pole pruner and mobile elevating work platform for canopy access. Three-five leaf samples were collected from the branches and 30 leaf discs, 1 cm in diameter, were punched out from the leaves avoiding the midrib and immediately stored in portable liquid N tank. The samples were transported to the laboratory and stored in a deep freezer at -80°C for measurement of LMA, leaf N and carbon (C) concentration, and N allocation.

In order to see the relationship between leaf and soil N concentration, soil sampling was conducted randomly after two categorizations based on sampled trees' location and DBH distribution. First, the sampling location was divided into 3 and 4 regions for *R. pseudoacacia* and *C. controvesa*, respectively. The number of location-dependent categories was different due to the differences in the number of individual trees sampled per species and their spatial distribution. Second, the DBH was divided into three categories; < 6 cm, 6 - 15 cm and > 15cm. After these two classification, one tree per each species from each category was randomly selected and three soil samples were collected from each trees, making total of 63 samples (= 27 + 36). Samples were collected from three vertices of an equilateral triangle connecting the center point of crown radius at the depth of 10 - 15 cm in June 2017. Total N and available N (NH_4^+ and NO_3^-) concentrations were measured by using the

Kjeldahl method (Bremner 1960) at National Instrumentation Center for Environmental Management (NICEM, Seoul National University, Seoul, Korea).

2.3. Leaf gas exchange

Gas exchange measurements were conducted on three leaves from seven individual trees of each species before leaf discs were made. The cut branches were immediately recut under water and used for gas exchange measurements (Huxman et al. 2003; Koike 1986; Potts et al. 2017; Sendall and Reich 2013). Gas exchange measurements were conducted on three leaves from seven individual trees of each species using a portable photosynthesis measurement system (LI-6400, LI-COR, Lincoln, NE, USA). All of the measurements were carried out during in the morning or late afternoon after the symptoms of water stress are disappeared. Leaf photosynthesis (A) response curves to controlled internal CO_2 concentration (C_i) were established at a leaf temperature of 25°C and under high irradiance (1200 μmol m^{-2} s^{-1}). The CO_2 concentration values for A/C_i curve were changed in the following order: 400, 300, 200, 100, 75, 50, 0, 400, 600, 800, 1000, 1200, 1400 μmol m^{-2} s^{-1}. Data were fitted to version 2.0 of the curve-fitting model described by Sharkey (2016) to estimate the maximum carboxylation rate (V_{Cmax}), maximum electron transport rate (J_{max}).

2.4. Crown exposure

In order to determine whether other factors other than tree size effect on leaf traits and N allocation, crown exposure on each sampled tree was scored qualitatively according to Thomas (2010). The categories were divided into 9 from under shadow as 1 to fully exposed to the sun as 5 with 0.5 interval as follows: 1, trees without direct light either vertically or laterally; 1.5, trees with low lateral light exposure; 2, trees with medium lateral light exposure; 2.5, trees with high lateral light exposure; 3, co-dominant trees with < 50% of upper crown exposed to vertical light; 3.5, co-dominant trees with > 50% of upper crown exposed to vertical light; 4, dominant trees receiving full vertical and some lateral light, < 50% exposed within 90° inverted cone encompassing the crown; 4.5, dominant trees receiving full vertical and considerable lateral light, > 50% exposed within 90° inverted cone encompassing the crown; 5, emergent trees with crown completely exposed.

2.5. Chemical assay

2.5.1. Determination of LMA, leaf N and C

Ten leaf discs were dried at 70°C for a minimum of 72 hours before leaf dry mass was measured. LMA was then calculated as grams dry mass meter^{-2}. Dried foliar samples were ground to a fine powder using a homogenizer (FastPrep-24, MP Biomedicals, Solon, OH, USA) and total N and C were determined by a CHNS-Analyzer Flash EA 1112 (Thermo Electron Corporation, USA) at NICEM.

2.5.2. Determination of leaf N content in Rubisco

N_R was measured according to Hikosaka and Shigeno (2009) with modifications. One leaf disc was powdered using a homogenizer (FastPrep-24, MP Biomedicals, Solon, OH) and homogenized in 1 mL 50 mM Tris HCl buffer (pH 7.4) containing 0.1 mM & 0.2mM Ethylenediaminetetraacetic acid for *R.pseudoacacia* and *C.controversa*, respectively, 1% (w/v) Polyvinyl-polypyrrolidone, 0.1 mM Phenylmethlsulfonyl fluoride, and 0.2% (v/v) Triton X-100. The homogenate was centrifuged at 15,000 g for 30 min at 4°C and the supernatant was applied to sodium dodecyl sulfate (SDS; a detergent) polyacrylamide gel electrophoresis (SDS-PAGE) for determination of Rubisco content (Hikosaka et al. 1998). N in Rubisco was calculated assuming that N concentration in Rubisco is 16% (Hikosaka and Terashima 1995).

2.5.3. Determination of leaf N content in bioenergetics

N_B was estimated from gas exchange characteristics. N in bioenergetics is proportional to J_{max}, where the ratio of J_{max} to the cytochrome *f* content is 156 mmol mol^{-1} s^{-1} (Niinemets and Tenhunen 1997) and N in bioenergetics per unit cytochrome *f* is 9.53 mol $mmol^{-1}$ (Hikosaka and Terashima 1995).

2.5.4. Determination of leaf N content in chlorophyll

Chlorophyll was extracted with the dimethylsulphoxide (DMSO) method (Barnes et al. 1992; Hiscox and Israelstam 1979; Tait and Hik 2003). Two leaf discs were incubated in a brown glass vial with 5 mL of DMSO in a

water bath at 65°C for 6 hours in the dark. When the extractions were complete, samples were transferred to disposable polystyrene cuvettes. Absorbance of both blank (pure DMSO) and samples was measured at 649 and 665 nm with a spectrophotometer (wavelength range 190 – 1100 nm, spectral band pass width < 1nm, wavelength accuracy < ± 0.5 nm; Optizen 2120 UV, Mecasys, Korea). Total chlorophyll (chlorophyll a + b) contents per unit leaf area were estimated using Wellburn's (1994) equation for DMSO and spectrophotometers with a resolution range of 0.1 – 0.5 nm: total chlorophyll ($\mu g ml^{-1}$) = 21.44 A_{649} + 5.97 A_{665}. The chlorophyll concentrations of the extracts calculated from this equation were then converted into leaf chlorophyll contents per unit area. N_H was calculated assuming 37.1 mol mol^{-1} chlorophyll (Evans 1989b).

2.5.5. Determination of leaf N content in cell wall

N_W was measured according to Hikosaka and Shigeno (2009). Ten leaf discs were powdered using the same homogenizer and homogenized in 1 mL 100 mM Bicine buffer (pH 8.0) with 5 mM dithiothreitol, 2% SDS, and 1% polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 g for 30 min at 4°C. The pellet was washed with the same buffer again and then washed with water to remove SDS. To remove chlorophylls completely, strong solvent PAW (phenol:acetic acid:water = 2:1:1, w:v:v; Fry 1988) was used. The pellet was treated with PAW three times more (1 hour, overnight, and 1 hour, respectively) to completely removed nitrogenous compounds other than cell wall proteins (Hikosaka and Shigeno 2009). The pellet was then washed with ethanol until there was no smell of phenol. The pellet was then dried at 70°C for more than

3 days. The dry mass was measured and considered as cell wall mass, and cell wall N was determined by measuring N concentration of the dried pellet at NICEM.

2.6. Statistical analysis

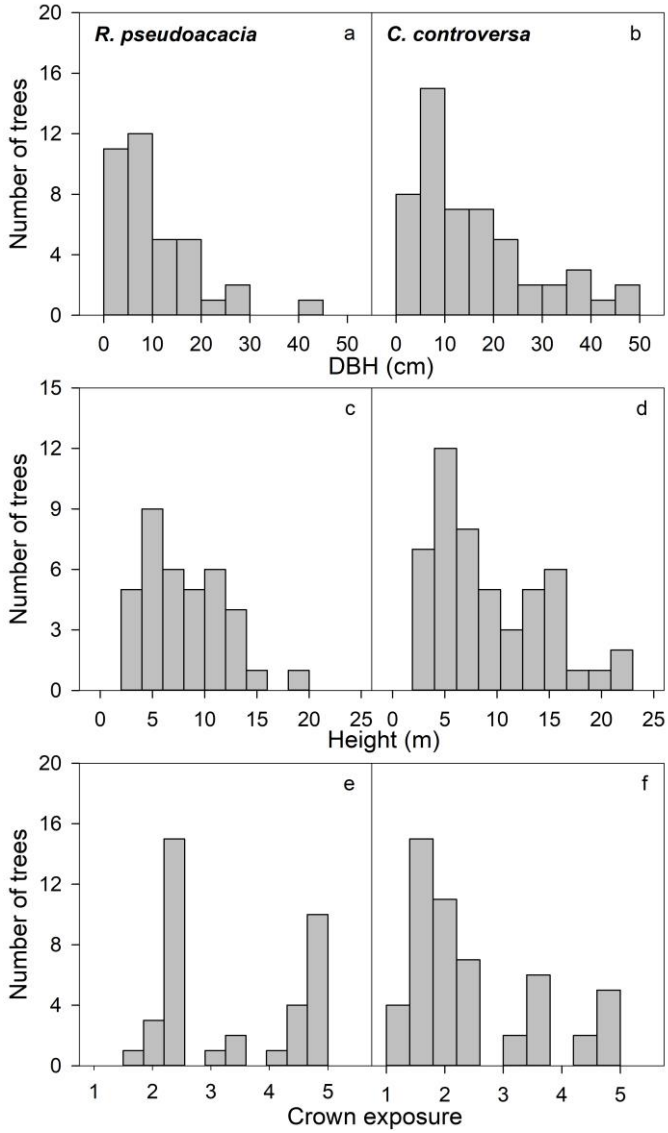
The comparison of mean soil and leaf traits between *R. pseudoacacia* and *C. controversa* were evaluated with independent *t*-tests. In order to make even distribution along tree DBH, all variables were $\log_{10}$-$\log_{10}$ transformed in size-trait relationships to improve normality and reduce heteroscedasticity. The relationship between tree DBH and leaf morphological and physiological properties of leaves were analyzed using linear and second-order polynomial regression analysis using SigmaPlot 12.5 (Systat Software Inc., Chicago, IL, USA). The effects of DBH and light exposure on traits were tested simultaneously, by including DBH and DBH^2 (when applicable) and crown exposure in a multiple regression model. The difference between regression lines was tested by analysis of variance comparing the sum of separate regressions with the joint regression (Quenouille 2014). Statistical analyses were performed using R software (ver. 3.3.2, available from https://www.R-project.org).

3. Results

3.1. Distribution of DBH, height and crown exposure

The mean DBH sampled of *C. controversa* was 15.60 ± 1.39 cm (1.3 – 41.3 cm DBH) and it was larger than that of *R. pseudoacacia* which was 10.32 ± 1.73 cm (0.8 – 48.8 cm DBH) ($P = 0.02$) (Figure 2a and b). The difference in mean DBH was caused by the number of large trees. There was only one *R. pseudoacacia* tree larger than 30 cm DBH which composed of 2.7% of their sample size. However, the 15.38% of *C. controversa* had DBH greater than 30 cm. Both distributions were strongly skewed to the right (skewness = 1.75 and 1.04 for *R. pseudoacacia* and *C. controversa*, respectively). Similar to the DBH, the mean height of the *R. pseudoacacia* was smaller than *C. controversa*, but there was no significant difference (8.11 ± 0.62 m (2.6 – 18.6 m) vs 9.15 ± 0.76 m (1.9 - 23 m), *R. pseudoacacia* and *C. controversa*, respectively, Figure 2c and d).

The distributions of the crown exposure indices showed two distinct patterns (Figure 2e and f). Crown exposure indices of *R. pseudoacacia* had binomial pattern which had two peaks at 2.5 and 5, representing crown exposed to high lateral light and to the full light. In contrast, *C. controversa* had the inverse J shape of crown exposure indices with a peak at 1.5.



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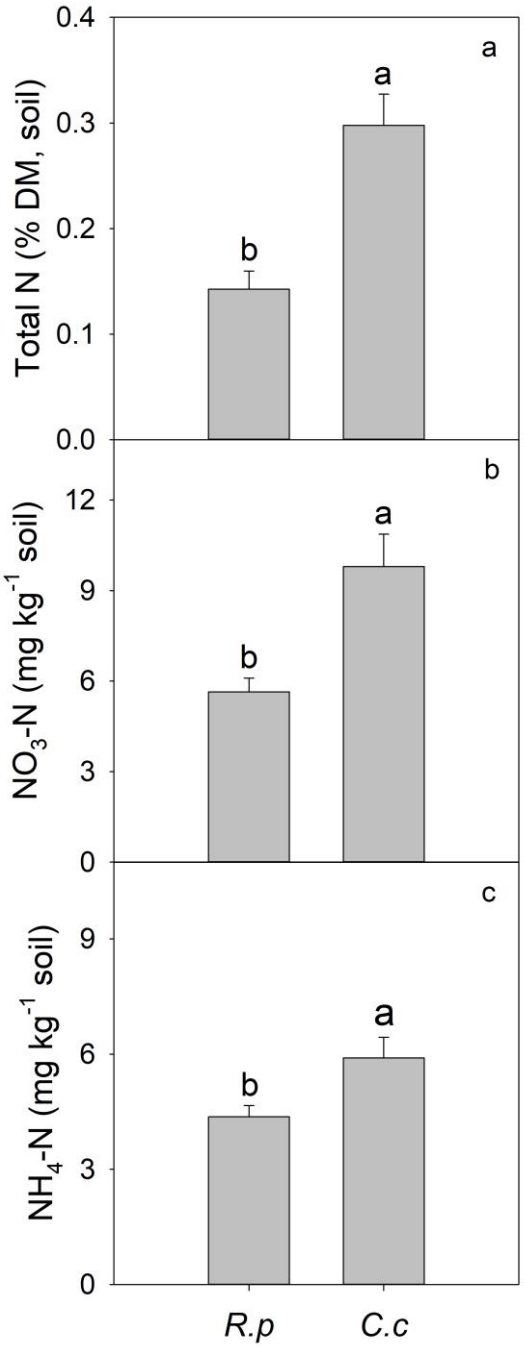
3.2. Soil N concentration

Soil total N concentration were higher under the crown of *C. controversa* than that of *R. pseudoacacia* (0.14 ± 0.02% and 0.3 ± 0.03% for *R. pseudoacacia* and *C. controversa*, respectively, $P < 0.0001$, Figure 3a). Similar to soil total N concentration, two types of available soil N, ammonium and nitrate, were about 35 and 75% higher under *C. controversa* than that of *R. pseudoacacia* (maximum $P = 0.016$, Figure 3b and c).

All soil N had no significant relationship with tree DBH and leaf total N which implies the future relationships between leaf physiological traits and DBH were not affected by soil N concentration (see Appendix 1).



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3.3. Comparisons of leaf morphological and physiological traits

LMA of two tree species was not significantly different ($P = 0.06$) even though *C. controversa* had ~ 14% higher LMA of 67.3 g m^{-2} than that of *R. pseudoacacia* (Figure 4a). In contrast, the mean total N and C:N ratio were significantly different between tree species (maximum $P < 0.001$). Total N of *R. pseudoacacia*, which was 3.53 ± 0.06%, was higher than that of *C. controversa*, which was 2.35 ± 0.05% ($P < 0.0001$, Figure 4b). On the other hand, *R. pseudoacacia* had C:N ratio smaller than that of *C. controversa* ($P < 0.0001$, Figure 4c). *R. pseudoacacia* had the C:N ratio of 13.85 ± 0.23 and *C. controversa* had 20.24 ± 0.49.

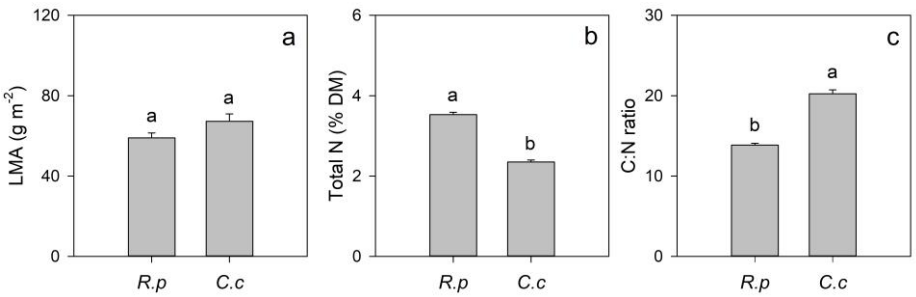

Figure 4. Differences in (a) LMA (g m^{-2}) , (b) total N concentration (in % of dry mass, DM), and (c) C:N ratio of leaf between *R. pseudoacacia* (*R. p*) and *C.controversa* (*C. c*). The different letters within a panel denote significant differences between group mean values ($P < 0.05$). Error bars represent the standard error.

V_{Cmax} and J_{max} of *R. pseudoacacia* were about 1.9 times and 1.4 times greater than those of *C. controversa*, and these differences were significant (maximum $P < 0.001$, Figure 5). The V_{Cmax} values of *R. pseudoacacia* and *C. controversa* were 75.28 ± 5.15 μmol m^{-2} s^{-1} and 39.58 ± 3.75 μmol m^{-2} s^{-1} (Figure 5a), meanwhile their J_{max} values were 106.77 ± 6.12 μmol m^{-2} s^{-1}, 73.74 ± 4.07 μmol m^{-2} s^{-1}, respectively (Figure 5b).

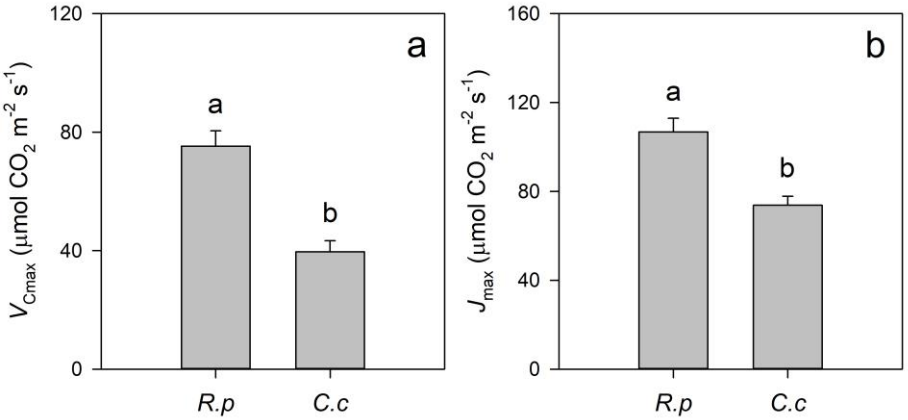

Figure 5. Differences in (a) maximum carboxylation rate (V_{Cmax}) and (b) maximum electron transport rate (J_{max}) between *R. pseudoacacia* (*R. p*) and *C.controversa* (*C. c*). The different letters within a panel denote significant differences between group mean values ($P < 0.05$). Error bars represent the standard error.

3.4. Influence of crown exposure

LMA varied significantly as a function of crown exposure while holding DBH constant in both species (Table 1). In *C. controversa*, LMA, N_R, V_{Cmax}, and J_{max} showed net effect of DBH when controlling for crown exposure. In contrast, different leaf traits, LMA, N_L, N_B, and N_W, showed the net effect of DBH in *R. pseudoacacia*.

Table 1. Results of multiple regression analysis of leaf traits variation as a function of tree size (DBH) and crown exposure. Parameter estimates and results (*P* values) in *R. pseudoacaia* and *C. controversa* are shown. Second-order polynomial terms were included in the analysis when traits exhibit significant DBH^2. *P* values in bold are significant at $P < 0.05$.

Variable	Term	*R. pseudoacacia*		*C. controversa*	
		Parameter	*P*-value	Parameter	*P*-value
LMA	Exposure	0.036	**0.013**	0.025	**0.253**
	DBH	0.177	**0.001**	0.28	**0.0001**
Total N	Exposure	-0.006	0.246	0.01	0.375
	DBH	-0.21	**0.03**	0.084	0.404
	DBH^2	0.089	0.089	-0.109	0.054
N_R	Exposure	-0.018	0.449	0.031	0.284
	DBH	-0.117	0.168	0.745	**0.006**
	DBH^2	-	-	-0.498	**0.001**
N_H	Exposure	-0.009	0.378	0.005	0.831
	DBH	-0.071	0.053	-0.033	0.869
	DBH^2	-	-	-0.111	0.315
N_B	Exposure	-0.002	0.915	0.02	0.763
	DBH	-0.164	**0.026**	-0.213	0.096
N_W	Exposure	0.002	0.909	0.029	0.430
	DBH	-0.794	**0.019**	-0.389	0.245
	DBH^2	0.463	**0.013**	0.222	0.232
V_{Cmax}	Exposure	0.033	0.279	0.079	0.53
	DBH	0.072	0.424	3.026	**0.038**
	DBH^2	-	-	-1.384	0.059
J_{max}	Exposure	0.032	0.243	0.067	0.437
	DBH	0.02	0.802	1.956	**0.048**
	DBH^2	-	-	-0.856	0.082

3.5. The size-dependency of leaf morphological and physiological traits

LMA increased linearly with tree size in both species, and the relationship between LMA and tree DBH was not different among species (Figure 6a). On average, LMA increased from 30.9 to 97.2 g m^{-2} as DBH increase from 1 to 50 cm (LMA = 0.29 DBH + 1.49, $R^2 = 0.506$ and $P < 0.0001$). In contrast, leaf N content decreased with DBH in both species (Figure 6b). The relationship of leaf total N and tree DBH were best explained by second-order polynomial functions. However, there were difference in declining patterns; in *C. controversa*, leaf total N remained constant ~2.4% when the tree size is small and began to decrease from about 5 cm DBH, while in *R. pseudoacacia*, leaf total N peaked in the smallest tree sizes (1.9 cm) with ~4% and declined immediately up to 15 cm and then remained constant.

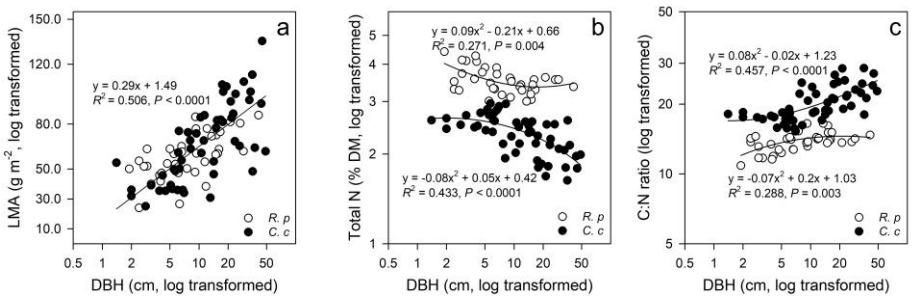

Figure 6. Leaf morphological and physiological traits as a function of tree DBH for *R. pseudoacacia* (*R. p*) (circle) and *C. controversa* (*C. c*) (filled circle). Leaf traits include (a) LMA, (b) leaf total N, (c) C:N ratio. Trend lines represent leaf traits-DBH relationships based on regression analysis performed on log-log transformed data.

The ratio of C to N increased with tree DBH in both species, but the pattern was the opposite of leaf total N, having initial increase and reaching asymptote at 5 cm for *R. pseudoacacia* vs slow and gradually greater increase with DBH for *C. controversa* (Figure 6c).

V_{Cmax} and J_{max} on a leaf area basis showed hump-shaped pattern relative to tree size only in *C. controversa* (Figure 7a and b). However, V_{Cmax} and J_{max} of *R. pseudoacacia* had no relationship with the tree size.

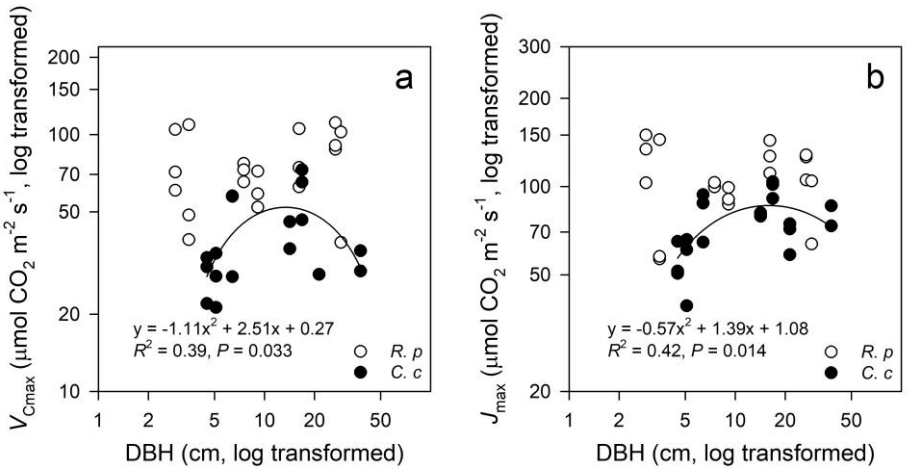

Figure 7. Relationships between (a) V_{Cmax} and (b) J_{max} and tree DBH for *R. pseudoacacia* (*R. p*) (circle) and *C. controversa* (*C. c*) (filled circle). Trend lines represent leaf photosynthetic parameters-DBH relationships based on regression analysis performed on log-log transformed data.

3.6. N allocation characteristics

Figure 8 shows the N concentration allocated to Rubisco, bioenergetics, light harvesting, and cell wall. On average, N fixing *R. pseudoacacia* had higher N concentration in Rubisco, bioenergetics, and cell wall than *C. controversa* (minimum $P = 0.01$). N_R were 0.44 ± 0.03 and 0.26 ± 0.01%, N_B were 0.13 ± 0.01 and 0.1 ± 0.01%, and N_W were 0.78 ± 0.04 and 0.14 ± 0.01% for *R. pseudoacacia* and *C. controversa*, respectively (Figure 8a, b and d). However, *C. controversa* had higher N_H than *R. pseudoacacia* (0.57 ± 0.02 and 0.66 ± 0.03%, for *R. pseudoacacia* and *C. controversa*, respectively, Figure 8c).

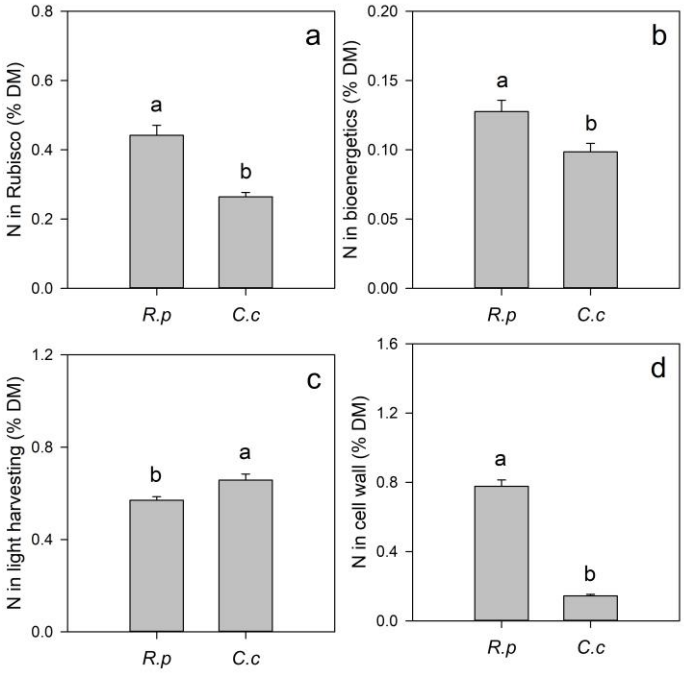

Figure 8. Differences in (a) N in Rubisco (in % of dry mass, DM), (b) N in bioenergetics (% DM), (c) N in light harvesting (% DM), and (d) N in cell wall (% DM) between *R. pseudoacacia* (*R. p*) and *C. controversa* (*C. c*). The different letters within a panel denote significant differences between group mean values ($P < 0.05$). Error bars represent the standard error.

Figure 9 shows the change of N_R, N_B, N_H, and N_W with tree DBH within and between species. N_R showed 'hump-shaped' relationship with DBH in *C. controversa*, reaching maximum value of 0.303% at ~ 6 cm and minimum value of 0.137% at 50 cm ($P < 0.0001$, Figure 9a), while that of *R. pseudoacacia* showed generally decreasing pattern, which was not significant ($P = 0.086$). In the meantime, N_B of both species linearly decreased with DBH ($P = 0.022$, $P = 0.043$, for *R. pseudoacacia* and *C. controversa*, Figure 9b). Similar to N_B, N_H in both species decreased with DBH. However, N_H of *C. controversa* drastically decreased across tree DBH, while that of *R. pseudoacacia* showed weak negative relationship with tree DBH ($P < 0.0001$, $P = 0.02$ for *C. controversa*

and *R. pseudoacacia*, respectively, Figure 9c). Unlike other three components, N_W showed 'U-shaped' relationship with DBH, reaching their minimum values at ~ 6 cm in both species (P = 0.03, P = 0.025, for *R. pseudoacacia* and *C. controversa*, Figure 9d).

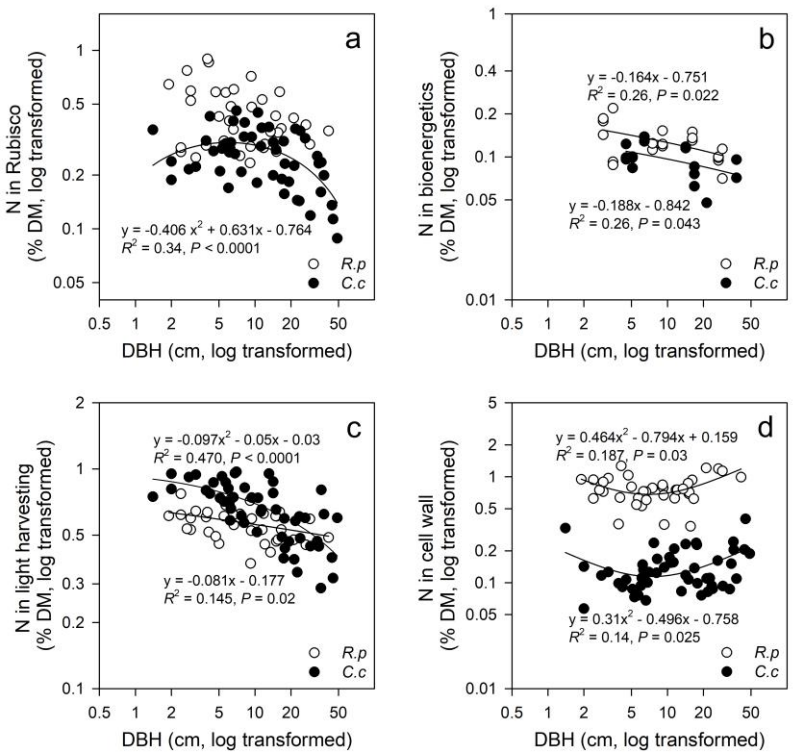

Figure 9. Size-dependent changes on N allocation characteristics (in % of dry mass, DM) for *R. pseudoacacia* (*R. p*) (circle) and *C. controversa* (*C. c*) (filled circle). (a) N in Rubisco, (b) N in bioenergetics, (c) N in light harvesting, and (d) N in cell wall for each species. Trend lines represent leaf N allocation characteristics-DBH relationships based on regression analysis performed on log-log transformed data.

4. Discussion

Two species with different life history strategies presented differences in key leaf trait as well as leaf N allocation. Moreover, those species-specific differences occurred in tree size-dependent change on leaf trait and N allocation characteristics.

4.1. Influence of soil N concentration on leaf traits

Although it is generally known that the tree leaf N concentration depends on soil N, my study did not show significant relationship between them (see Appendix 1). This indicates the soil N, when used alone, is inadequate to characterize N supply in relation to the leaf (Ordonez et al. 2009). According to Chapin (1983), leaf N concentration is not only controlled by soil nutrient availability, but also efficiency and rate of nutrient uptake, nutrient partitioning within plant, and growth rates of the plant.

In addition, unexpectedly soil N concentration under *R. pseudoacacia*, which had higher leaf N than *C. controversa*, was lower than that of *C. controversa* (Figure 2). This lower soil N could be attributed to the location of *R. pseudoacacia*, which grows usually in road side due to their high affinity to light, was prone to high leaching, run off and soil erosion (Cierjacks et al. 2013; Vitkova et al. 2017; Vitkova et al. 2015). In contrast, *C. controversa*, which also happen to occur near forest road, but further inside, where forest floor development was progressed much more extensively, resulting in richer nutrient contents than under *R. pseudoacacia*.

4.2. Species-specific characteristics of leaf traits

In line with our expectation, *R. pseudoacacia* had high N content, low C:N ratio, and high photosynthetic parameters (V_{Cmax}, J_{max}). These findings are consistent with the characteristics of early successional species reported in several studies (Figure 4a and c, Figure 5a and b, Coste et al. 2005; Koike 1988; Matsuki and Koike 2006). For example, in Matsuki and Koike (2006), three early successional species, *Alnus hirsute*, *Betula maximowicziana* and *Betula platyphylla* var. *japonia* had about 3% of total N content and 16.6 of C:N ratio, while late successional species, *Carpinus cordata*, *Quercus mongolica* var. *grosseserrata* and *Acer mono* had about 1.6%, 26.7, respectively. In particular, a N-fixing species, *Alnus hirsute* had about 3.4% of total N, which was similar value in *R. pseudoacacia* (3.53%, Figure 4a). In addition, pioneer *Cecropia obtuse* displayed the largest mean of V_{Cmax} (55.3 μmol m^{-2} s^{-1}) among the study species with different ecological group in Coste et al. (2005). The V_{Cmax} value was lower than that of *R. pseudoacacia* (75.28 ± 5.15 μmol m^{-2} s^{-1}), but still higher than that of *C. controversa* (39.58 ± 3.75 μmol m^{-2} s^{-1}) (Figure 5a). These findings of *R. pseudoacacia* support their role as early successional species: they grow quickly and reproduce before competitors do (Matsuki and Koike 2006; Menge et al. 2008). LMA of *R. pseudoacacia* was marginally significantly lower than that of *C. controversa* (Figure 4b). High N content and low LMA of *R. pseudoacacia* have been reported in Tanaka-Oda et al. (2010) and Jin et al. (2011), showing characteristic of luxurious resource use and accompanying a high relative growth rate (Poorter et al. 2009).

4.3. Size-related changes in leaf traits

Changes of leaf N and C:N ratio across tree size showed non-linear patterns and the patterns of two species was opposite (Figure 6b and c). These patterns are similar to those observed in other findings (Martin and Thomas 2013; Sendall and Reich 2013; Thomas 2010). The results of Martin and Thomas (2013) identified that the relationship of leaf N and C:N ratio with tree size was better described using polynomial functions than simple linear regressions. Martin and Thomas (2013) hypothesize that mid- and late-successional tree species may predict vertical gradients in light as their crown reached the canopy. Because of a high 'costs of acclimation' for this, the species could have evolved a deterministic developmental program in which seedling leaves show shade-plant traits, but progressively have sun-plant traits as they grow (Martin and Thomas 2013). While *Dacryodes excels*, long-lived late successional species, showed distinct maxima in leaf total N at intermediate DBH (Martin and Thomas 2013), leaf total N content of *C. controversa*, mid-successional species, showed constant phase until about 5 cm in my study. It may be interpreted that late successional species show more shade-plant traits in the seedling phase than mid-successional species (Thomas 2010). On the contrary, leaf total N content in *R. pseudoacacia* which is early-successional species peaked in the smallest tree size, which is consistent with pattern in pioneer *Miconia mirabilis* in the results of Martin and Thomas (2013).

4.4. Leaf N allocation

The amounts of N allocated in photosynthetic and structural apparatus were all different between *R. pseudoacacia* and *C. controversa*. N-fixing *R. pseudoacacia* have more N content in each component having one exception of N allocated in light harvesting component (Figure 8). It is thought that *C. controversa* invests N much in chlorophyll as a species with shade tolerance (Poorter and Bongers 2006; Valladares and Niinemets 2008). However, light harvesting N content in *C. controversa* has a large variation with tree size resulting in that it is smaller than that of *R. pseudoacacia* for very large tree size (Figure 9c). However, N in Rubisco and light harvesting component of *R. pseudoacacia* decreased slowly across tree size probably due to sufficient light environment condition of the species (Figure 9a and 9c). Although very few data are available on N partitioning in different sized trees, these results are in accord with evidences of a lower fraction of N in the photosynthetic apparatus in older and larger trees (Juarez-Lopez et al. 2008; Merilo et al. 2009; Niinemets 2002) resulting size- dependent reduction in foliage photosynthetic potential (Figure 7b, Woodruff et al. 2009). While the amounts of N allocated in all photosynthetic apparatus decrease with tree size as a whole pattern, cell wall N content increase with tree size (Figure 9d). It was thought that the reasons of rigid leaves on older trees may be due to wind, herbivory (Patankar et al. 2011) and high light exposure.

5. Conclusion

Our research revealed two species (*R. pseudoacacia* and *C. controversa*) with different life history strategies had different amounts and patterns in leaf traits with their size. Furthermore, I evaluated the reason why two species had the opposite patterns in leaf N with tree size by quantifying the N content of different parts of individual cell. Both species had similar allocation patterns for N in bioenergetics and N in cell wall, which showed linear decrease and U shape with size. N in Rubisco and N in light harvesting of *R. pseudoacacia* decreased linearly with size. However, *C. controversa* invested large portion of N to light harvesting system to capture maximum light while they were small and gradually reduced their investment until mid-size and reduced rapidly as their crown reached the canopy. As compensation, N investment into Rubisco was lower at the beginning and then increased up to mid-size followed by sharp reduction. In addition, these differences in allocation were confirmed as the net effect of tree size. In conclusion, results indicate that leaf traits change intra- and inter- specifically with increased tree DBH, implying that trees alter their N allocation within photosynthetic and structural apparatus in leaf according to the life history strategies as they grow.

References

Adler PB, Salguero-Gomez R, Compagnoni A, Hsu JS, Ray-Mukherjee J, Mbeau-Ache C, Franco M (2014) Functional traits explain variation in plant life history strategies (vol 111, pg 740, 2014). Proceedings of the National Academy of Sciences of the United States of America. 111:10019-10019.

Barnes JD, Balaguer L, Manrique E, Elvira S, Davison AW (1992) A Reappraisal of the Use of DMSO for the Extraction and Determination of Chlorophylls a and b in Lichens and Higher-Plants. Environmental and Experimental Botany. 32:85-100.

Bond BJ (2000) Age-related changes in photosynthesis of woody plants. Trends Plant Sci. 5:349-53.

Botkin DB, Janak JF, Wallis JR (1972) Rationale, Limitations, and Assumptions of a Northeastern Forest Growth Simulator. Ibm Journal of Research and Development. 16:101-&.

Bremner J (1960) Determination of nitrogen in soil by the Kjeldahl method. The Journal of Agricultural Science. 55:11-33.

Buol SW, Southard RJ, Graham RC, McDaniel PA (2011) Soil genesis and classification. John Wiley & Sons.

Chapin FS (1983) Direct and Indirect Effects of Temperature on Arctic Plants. Polar Biology. 2:47-52.

Cho, JH, Hong, SG, Kim, JJ (2000) Effects of Shading on Growth of 1-year-old *Cornus controversa* HEMSL. Seedlings). Journal of Korea forestry energy, 19.1: 20-29. (In Korean).

Choi, DY, Chang, CS, Kim, JY, Kim, H, Min, WK, Kang UT (2001). Flora of province Gyonggi-do. The Arboretum, Seoul National University. Bulletin of the Seoul National University Arboretum, 21, 25-76. (In Korean).

Cierjacks A, Kowarik I, Joshi J, Hempel S, Ristow M, von der Lippe M, Weber E (2013) Biological Flora of the British Isles: *Robinia pseudoacacia*. Journal of Ecology. 101:1623-1640.

Cornelissen J (1993) Seedling growth and morphology of the deciduous tree Cornus controversa in simulated forest gap light environments in subtropical China. Plant Species Biology. 8:21-27.

Coste S, Roggy JC, Imbert P, Born C, Bonal D, Dreyer E (2005) Leaf photosynthetic traits of 14 tropical rain forest species in relation to leaf nitrogen concentration and shade tolerance. Tree Physiol. 25:1127-37.

Evans JR (1989a) The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences and control. Photosynthesis

Evans JR (1989b) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia. 78:9-19.

Field C, Mooney H (1986) On the economy of plant form and function: adaptative patterns of energy capture in plants

Fredericksen TS, Steiner KC, Skelly JM, Joyce BJ, Kolb TE, Kouterick KB, Ferdinand JA (1996) Diel and seasonal patterns of leaf gas exchange and xylem water potentials of different-sized *Prunus serotina* Ehrh trees. Forest Science. 42:359-365.

Fry SC (1988) The growing plant cell wall: chemical and metabolic analysis.

Longman Group Limited.

Garnier E, Cortez J, Billes G, Navas ML, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellmann A, Neill C, Toussaint JP (2004) Plant functional markers capture ecosystem properties during secondary succession. Ecology. 85:2630-2637.

Gower ST, McMurtrie RE, Murty D (1996) Aboveground net primary production decline with stand age: potential causes. Trends Ecol Evol. 11:378-82.

Guan LL, Wen DZ (2011) More nitrogen partition in structural proteins and decreased photosynthetic nitrogen-use efficiency of *Pinus massoniana* under in situ polluted stress. J Plant Res. 124:663-73.

Hikosaka K (2005) Nitrogen partitioning in the photosynthetic apparatus of *Plantago asiatica* leaves grown under different temperature and light conditions: similarities and differences between temperature and light acclimation. Plant Cell Physiol. 46:1283-90.

Hikosaka K, Hanba YT, Hirose T, Terashima I (1998) Photosynthetic nitrogen-use efficiency in leaves of woody and herbaceous species. Functional Ecology. 12:896-905.

Hikosaka K, Shigeno A (2009) The role of Rubisco and cell walls in the interspecific variation in photosynthetic capacity. Oecologia. 160:443-51.

Hikosaka K, Terashima I (1995) A Model of the Acclimation of Photosynthesis in the Leaves of C3 Plants to Sun and Shade with Respect to Nitrogen Use. Plant Cell and Environment. 18:605-618.

Hiscox JD, Israelstam GF (1979) Method for the Extraction of Chlorophyll from Leaf Tissue without Maceration. Canadian Journal of Botany-Revue Canadienne De Botanique. 57:1332-1334.

Hobbs RJ (2000) Land-use changes and invasions. Invasive species in a changing world:55-64.

Hobbs RJ, Huenneke LF (1992) Disturbance, Diversity, and Invasion - Implications for Conservations. Conservation Biology. 6:324-337.

Hoch G, Popp M, Korner C (2002) Altitudinal increase of mobile carbon pools in Pinus cembra suggests sink limitation of growth at the Swiss treeline. Oikos. 98:361-374.

Huxman TE, Turnipseed AA, Sparks JP, Harley PC, Monson RK (2003) Temperature as a control over ecosystem CO_2 fluxes in a high-elevation, subalpine forest. Oecologia. 134:537-46.

Jin TT, Liu GH, Fu BJ, Ding XH, Yang L (2011) Assessing adaptability of planted trees using leaf traits: A case study with *Robinia pseudoacacia* L. in the Loess Plateau, China. Chinese Geographical Science. 21:290-303.

Juarez-Lopez FJ, Escudero A, Mediavilla S (2008) Ontogenetic changes in stomatal and biochemical limitations to photosynthesis of two co-occurring Mediterranean oaks differing in leaf life span. Tree Physiol. 28:367-74.

Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. Nature. 428:851-4.

Koike T (1986) A method for measuring photosynthesis with detached parts of

deciduous broad-leaved trees in Hokkaido. Journal of the Japanese Forestry Society. 68:425-428.

Koike T (1988) Leaf structure and photosynthetic performance as related to the forest succession of deciduous broad-leaved trees1. Plant Species Biology. 3:77-87.

Kolb TE, Stone JE (2000) Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine-oak forest. Tree Physiology. 20:1-12.

Korea Forest Service (KFS) (1997) Present status and use of black locust plantation. Korea Forest Service, Seoul, unpublished data (in Korean).

Kowarik I (1999) Neophytes in Germany: Quantitative overview, introduction and dispersal pathways, ecological consequences and open questions. Texte des Umweltbundesamtes Berlin. 18:12-36.

Lambers H, Poorter H (1992) Inherent Variation in Growth-Rate between Higher-Plants - a Search for Physiological Causes and Ecological Consequences. Advances in Ecological Research. 23:187-261.

Lavorel S, Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional Ecology. 16:545-556.

Lundholm J, Tran S, Gebert L (2015) Plant functional traits predict green roof ecosystem services. Environ Sci Technol. 49:2366-74.

Martin AR, Thomas SC (2013) Size-dependent changes in leaf and wood chemical traits in two Caribbean rainforest trees. Tree Physiol. 33:1338-53.

Matsuki S, Koike T (2006) Comparison of leaf life span, photosynthesis and defensive traits across seven species of deciduous broad-leaf tree seedlings. Ann Bot. 97:813-7.

McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol. 21:178-85.

Meffe G, Carroll C, Pimm S. 1997. Community-and ecosystem-level conservation: species interactions, disturbance regimes, and invading species. Sinauer Associates: Sunderland, pp 236-267.

Menge DN, Levin SA, Hedin LO (2008) Evolutionary tradeoffs can select against nitrogen fixation and thereby maintain nitrogen limitation. Proc Natl Acad Sci U S A. 105:1573-8.

Merilo E, Tulva I, Raim O, Kukit A, Sellin A, Kull O (2009) Changes in needle nitrogen partitioning and photosynthesis during 80 years of tree ontogeny in Picea abies. Trees-Structure and Function. 23:951-958.

Niinemets U (2002) Stomatal conductance alone does not explain the decline in foliar photosynthetic rates with increasing tree age and size in *Picea abies* and *Pinus sylvestris*. Tree Physiology. 22:515-535.

Niinemets Ü, Tenhunen J (1997) A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species Acer saccharum. Plant, Cell & Environment. 20:845-866.

Onoda Y, Hikosaka K, Hirose T (2004) Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. Functional Ecology. 18:419-425.

Ordonez JC, van Bodegom PM, Witte JPM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography. 18:137-149.

Patankar R, Thomas SC, Smith SM (2011) A gall-inducing arthropod drives declines in canopy tree photosynthesis. Oecologia. 167:701-9.

Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis (vol 182, pg 565, 2009). New Phytologist. 183:1222-1222.

Poorter L, Bongers F (2006) Leaf traits are good predictors of plant performance across 53 rain forest species. Ecology. 87:1733-43.

Potts D, Minor R, Braun Z, Barron-Gafford G, Tissue D (2017) Photosynthetic phenological variation may promote coexistence among co-dominant tree species in a Madrean sky island mixed conifer forest. Tree Physiology:1-10.

Quenouille MH (2014) Introductory statistics. Elsevier.

Rijkers T, Pons TL, Bongers F (2000) The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. Functional Ecology. 14:77-86.

Sendall KM, Reich PB (2013) Variation in leaf and twig CO_2 flux as a function of plant size: a comparison of seedlings, saplings and trees. Tree Physiol. 33:713-29.

Sharkey TD (2016) What gas exchange data can tell us about photosynthesis. Plant Cell Environ. 39:1161-3.

Showalter AM (1993) Structure and function of plant cell wall proteins. Plant Cell. 5:9-23.

Sohng J, Han AR, Jeong MA, Park Y, Park BB, Park PS (2014) Seasonal Pattern of Decomposition and N, P, and C Dynamics in Leaf litter in a Mongolian Oak Forest and a Korean Pine Plantation. Forests. 5:2561-2580.

Tait MA, Hik DS (2003) Is dimethylsulfoxide a reliable solvent for extracting chlorophyll under field conditions? Photosynth Res. 78:87-91.

Takashima T, Hikosaka K, Hirose T (2004) Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous Quercus species. Plant Cell and Environment. 27:1047-1054.

Tanaka-Oda A, Kenzo T, Koretsune S, Sasaki H, Fukuda K (2010) Ontogenetic changes in water-use efficiency (delta C-13) and leaf traits differ among tree species growing in a semiarid region of the Loess Plateau, China. Forest Ecology and Management. 259:953-957.

Thomas SC (2010) Photosynthetic capacity peaks at intermediate size in temperate deciduous trees. Tree Physiol. 30:555-73.

Thomas SC, Winner WE (2002) Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. Tree physiology. 22:117-127.

Valladares F, Niinemets U (2008) Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences Annual Review of Ecology Evolution and Systematics, pp 237-257.

Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E

(2007) Let the concept of trait be functional! Oikos. 116:882-892.

Visser MD, Bruijning M, Wright SJ, Muller-Landau HC, Jongejans E, Comita LS, de Kroon H (2016) Functional traits as predictors of vital rates across the life cycle of tropical trees. Functional Ecology. 30:168-180.

Vitkova M, Muellerova J, Sadlo J, Pergl J, Pysek P (2017) Black locust (*Robinia pseudoacacia*) beloved and despised: A story of an invasive tree in Central Europe. Forest Ecology and Management. 384:287-302.

Vitkova M, Tonika J, Mullerova J (2015) Black locust - successful invader of a wide range of soil conditions. Sci Total Environ. 505:315-28.

Wellburn AR (1994) The Spectral Determination of Chlorophyll a and b, as Well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. Journal of Plant Physiology. 144:307-313.

Woodruff DR, Meinzer FC, Lachenbruch B, Johnson DM (2009) Coordination of leaf structure and gas exchange along a height gradient in a tall conifer. Tree Physiol. 29:261-72.

Appendix 1

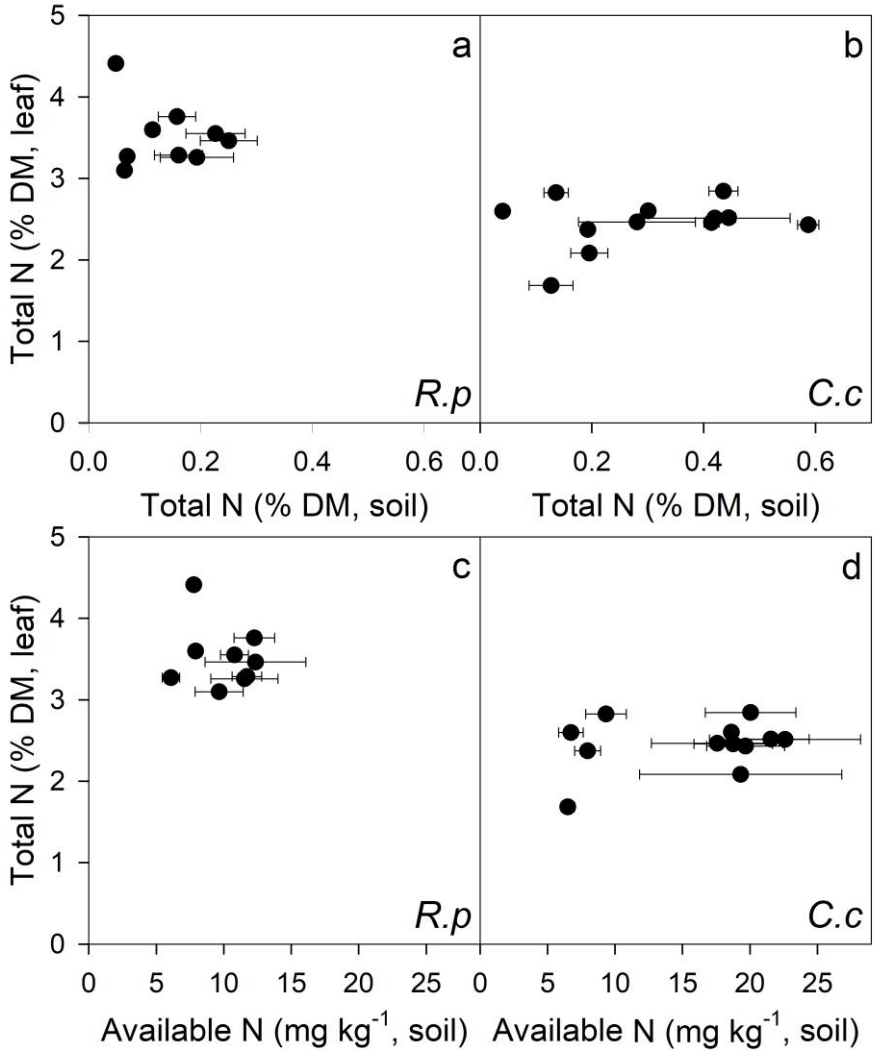

Figure 1. Relationships between (a, b) leaf total N (in % of dry mass, DM) and soil total N (in % of dry mass, DM) and relationships between (c, d) leaf total N and soil available N (mg kg^{-1}, nitrate N and ammonium N) for *R. pseudoacacia* (*R. p*) and *C. controversa* (*C. c*), respectively. Error bars represent the standard error.

Abstract in Korean

아까시나무와 층층나무의 흉고직경에 따른 잎의 생리적 특성과 질소 분배 특성의 변화

잎 수준에서의 특성을 이해하는 것은 산림생태계의 구조와 기능에 대한 수목의 반응, 또한 수목이 미치는 영향을 분석하고 예측하는 데 중요하다. 엽면적당 건중량, 잎의 전질소량과 같은 주요한 잎 특성은 수목의 생리적, 구조적 특성의 좋은 지표로 여겨진다. 그러나 잎의 특성이 수목의 성장에 따라 어떤 변화 양상을 보이는지에 대한 연구가 부족하여 수종별로 일반적인 변화 양상을 파악하기 어려우며, 현재 잎의 특성을 이용하는 대부분의 산림생태 모델에 이러한 변화가 적용되지 않고 있다. 본 연구에서는 서로 다른 생활사 전략을 가진 두 수종의 잎의 주요 생리적 특성을 나무의 크기에 따라 변화를 조사하고, 나아가 두 수종이 질소를 광합성기구와 구조적기구에 분배하는 양상과 변화를 함께 조사하였다. 본 연구는 경기도 태화산에 위치한 서울대학교 학술림에서 수행되었다. 태화산 내에 흔하게 분포하는 수종인 아까시나무(*Robinia pseudoacaia* L.)와 층층나무(*Cornus controversa* Hemsl.)를 연구대상 수종으로 선정하였다. 아까시나무는 빛 요구도가 큰 천이 초기단계의 수종이며 질소고정능력이 있고 층층나무는 천이 중기단계의 수종이면서 내음성이 강하다. 연구결과, 엽면적당 건중량은 두 수종 모두 흉고직경에 따

라 선형적으로 증가한 반면, 전질소량은 비선형적으로 감소하는 서로 다른 양상을 나타내었다. 아까시나무의 경우 광합성 능력이 나무의 크기가 증가함에 따라 변화하지 않았지만, 층층나무는 중간크기에서의 광합성 능력이 가장 높았다. 광합성 기구에 분배된 질소함량은 빛을 확보하는 기구에 분배된 질소를 제외하고 아까시나무가 층층나무보다 많았고, 흉고직경에 따라 통계적으로 유의한 경향을 나타내었다. 층층나무에서 루비스코 효소에 분배된 질소함량은 나무의 크기가 변하면서 크기가 작을 때 증가하다가 중간크기부터 감소하여 가장 높은 값을 갖는 지점을 갖는 양상을 보였다. 나머지 광합성 기구에 분배된 질소의 경우는 크기의 증가에 따라 단조 감소하였다. 세포벽 단백질에 분배하는 질소함량은 아까시나무가 층층나무보다 훨씬 더 많이 분배하는 것으로 나타났으며 두 수종 모두 나무의 크기에 따라 비슷한 경향성을 나타내어 성장 초기단계에서 감소하다가 증가하는 U자형 관계를 보였다. 결론적으로 수목은 성장하는 동안 생활사 전략에 따라 잎의 특징, 특히 잎 내 광합성과 구조 기관 내의 질소 분배량을 변화시켜 적응하는 것으로 판단된다.

주요어: 아까시나무, 층층나무, 잎의 기능적 특성, 크기 의존성, 엽면적당 건중량, 잎의 질소분배,

학 번: 2014-222820